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Mutation Frequencies of the Cytochrome *CYP2D6* Gene in Parkinson Disease Patients and in Families

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The frequencies of five mutations of the debrisoquine 4-hydroxylase (*CYP2D6*) gene (mutations *D6-A, B, C, D,* and *T*), corresponding to poor metabolizer (PM) phenotypes, were determined by restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) in 47 patients with Parkinson disease, and compared with the findings in 47 healthy controls. These mutant alleles were about twice as frequent among patients as in controls, with an approximate relative risk ratio of 2.12 (95% confidence interval, 1.41–2.62). There seem to be no significant differences in frequencies of mutant genotypes in patients among gender and modalities of response with levodopa therapy; but frequency of the mutations was slightly enhanced after age-at-onset of 60 years. Mutations *D6-B, D,* and *T* were detected in 7 patients belonging to 10 Parkinson pedigrees. © 1996 Wiley-Liss, Inc.

KEY WORDS: Parkinson disease; cytochrome P450 *CYP2D6* gene; poor metabolizer alleles; mutations *D6-A, B, C, D,* and *T;* studies in families

INTRODUCTION

As parkinsonism can be induced by chemical neurotoxins [Jenner et al., 1992] such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an attractive hypothesis of Parkinson disease etiology is one of genetic susceptibility to an environmental toxin. This has been explored by investigating the metabolism of debrisoquine, a drug which is hydroxylated by an enzyme of the cytochrome P450 system, specifically, debrisoquine 4-hydroxylase (*CYP2D6*). Impaired debrisoquine metabolism is found in 5–10% of Caucasian populations, as determined by a polymorphism of mutations at the *CYP2D6* gene causing a tendency towards poorer metabolism. Early reports on the incidence of poor metabolizer (PM) phenotypes in patients with Parkinson disease provided conflicting results, possibly because of limitations of the pharmacokinetic assay [Barbeau et al., 1985; Poirier et al., 1987; Benitez et al., 1990]. This problem can now be avoided by analyzing *CYP2D6* genotypes directly, and three more recent studies by genotypic studies [Armstrong et al., 1992; Smith et al., 1992; Kurth and Kurth, 1993] have indicated an excess of PM-associated genotypes in Parkinson disease patients as compared with controls.

We investigated the *CYP2D6* polymorphism by using restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) techniques that can be used to identify the *CYP2D6* wild-type (*wt*) allele and the five mutant alleles causing metabolically deficient (or partly deficient) phenotypes (corresponding to *CYP2D6 A, B, C, D,* and *T* variants) in Parkinson patients and controls. Characterization of these five mutants at the genomic level is as follows: the *D6-A* mutant allele contains a deletion in exon 5 of the gene, while the *D6-B* mutant allele contains multiple mutations leading to a splicing defect [Kagimoto et al., 1990]. The *D6-C* mutant allele involves deletion of 3 base pairs in exon 5, resulting in a single amino-acid deletion in the protein, and this product appears to function, but at a markedly reduced level [Tyndale et al., 1991]. In individuals with the *D6-D* mutation the entire gene is deleted, resulting in a 11.5-kb *XbaI* fragment [Gaedigk et al., 1991]. These four first mutant alleles have been shown to account for >95% of deficient metabolizers of debrisoquine [Heim and Meyer, 1990]. The more recently described *D6-T* mutant allele involves a single base deletion in exon 3, named T1795, resulting in a frame shift and generating a stop codon one codon after the deletion [Saxena et al., 1994].

The possibility of a genetic contribution to the pathogenesis of Parkinson disease has received increased support [Johnson, 1991], notably because several fami-

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lies with apparent autosomal-dominant inherited parkinsonism have been described. The *CYP2D6* polymorphism has been investigated in some patients with familial Parkinson disease [Gasser et al., 1994; Planté-Bordeneuve et al., 1994]. We have also investigated *CYP2D6* polymorphism in a group of 10 families containing more than one member with Parkinson disease, to assess whether *CYP2D6* mutations segregate with the disease.

PATIENTS AND METHODS

Patients and Controls

Blood samples were collected from 47 Parkinson disease patients seen at the Neurology Service, Reims, by one of us (J.-C.T.) between 1993–1994. Informed consent was a prerequisite for inclusion in the study, as approved by our local authorities. All patients were of "Champagne-Ardennes" regional origin, as determined by family history. Inclusion criteria [Hughes et al., 1992] concerned the presence of at least two of the following signs: resting tremor, bradykinesia, cogwheel rigidity, and postural reflex impairment; in our series, parkinsonism was not due to other known etiologies. Among the patients, 24 were male and 23 female. Efficiency of levodopa therapy, a requirement for diagnosis of Parkinson disease, was obtained for 30 patients, with others responding in a more fluctuating fashion. Average age-at-onset of all our Parkinson patients was 65.6 ± 9 years. The control population consisted of 47 unrelated individuals of the same regional origin, matched for sex and age.

Families

DNA was obtained from 19 Parkinson patients in 10 French families (see Fig. 1): two pedigrees from Reims, and eight from Paris [Planté-Bordeneuve et al., 1995], selected on the basis of an index case with clinically typical Parkinson disease and with at least one other affected relative.

Detection of *CYP2D6* Genotypes

Genomic DNA was extracted from 5 ml of blood using standard techniques. The *D6-D* mutation was detected after *Xba*I analysis by Southern hybridization with a full-length human *CYP2D6* cDNA probe [Skoda et al., 1988]. The most frequent *CYP2D6* mutation is the PM allele *D6-B*, a point-mutation at the 3′ splice site consensus sequence of intron 3 [Heim and Meyer, 1990], detected here by single PCR after restriction with *Mva*I; similarly, the *D6-T* mutation [Saxena et al., 1994] is also detected by single PCR, but after *Bsr*I restriction. *D6-A* and *D6-C* mutations [Broly et al., 1991] were detected by double PCR after restriction with *Hph*I.

RESULTS

Table I shows the distribution in patients with Parkinson disease and in controls of the six mutant *CYP2D6* genotypes which our assays could detect. Compared in such a manner (Table II), 25 of 47 cases (53%) and 33 of 47 controls (70%) had no detectable mutations (*wt/wt* genotype). The number of homozygous *D6-B* genotypes comprised 5 individuals with Parkinson disease vs. only 1 among controls, and the frequency of the *CYP2D6* mutant alleles was approximately doubled, from 15 of 94 control chromosomes (16%) to 27 of 94 in cases (28.7%). The estimated odds ratio for homozygous/heterozygous *CYP2D6* mutants in Parkinson disease is therefore 2.12 (95% confidence interval, 1.41–2.62).

No statistical difference was observed between allelic frequencies in Parkinson disease in both sexes (12/48 chromosomes = 25% for males, and 15/46 chromosomes = 32.6 for females. No statistical difference existed between allelic frequencies for frank (21/60 chromosomes = 35%) and fluctuating (6/34 = 17.6%) responders under levodopa therapy. Although an increase of allelic frequencies was noticed from early-onset (age-at-onset below 60 years) Parkinson disease (6/30 chromosomes = 20%) to late-onset (age-at-onset ≥60 years) Parkinson disease (21/64 = 32.8%), this difference was not statistically significant.

Figure 1 shows that some *CYP2D6* mutant alleles are detected in several patients for some pedigrees: *D6-B* in pedigrees 1, 2, 4, 9, and 10, *D6-D* in pedigree 6, and *D6-T* in pedigree 4. At least one *CYP2D6* mutant allele was detectable in 7 Parkinson patients out of 19 studied, and in six pedigrees out of 10 tested.

DISCUSSION

This study demonstrates in a French group of Parkinson disease patients an association of *CYP2D6*-deficient alleles with Parkinson disease, resulting in an approximate odds ratio of 2 in patients carrying these alleles for poor debrisoquine metabolism. This result independently confirms other similar studies of English [Armstrong et al., 1992; Smith et al., 1992] populations with alleles *D6-A, B, D,* and *E,* and an American [Kurth and Kurth, 1993] population with allele *D6-B;* notably, in British subjects, a relative risk ratio for PM of 2.54 was found among 229 patients with Parkinson disease [Smith et al., 1992], a result similar to that of our study.

The numbers of patients homozygous and heterozygous for these mutant alleles were almost twice in our Parkinson disease patients; hence, our data do not favor a role for a protective effect of the normal allele, as suggested by Smith et al. [1992], but are more consistent with the findings of Armstrong et al. [1992] and of Kurth and Kurth [1993], which showed an increase in heterozygotes as well as in homozygotes for the mutations. Our explanation considers as a risk factor the phenotypic effect of the *D6wt/D6B* genotype (i.e., intermediate oxidative status).

As already shown for one German-Canadian Parkinson pedigree [Gasser et al., 1994] and some English pedigrees [Planté-Bordeneuve et al., 1994] by linkage analysis, several patients in some of these families segregate with *CYP2D6* mutant alleles. Our own study of French families establishes that three *CYP2D6* mutant alleles are detectable in 7 patients (of 19 studied) in our 10 pedigrees. However, in only one pedigree does a mutant allele appear in more than one affected member (pedigree 9), and in no pedigree is there a mutant allele in more than one generation. The value of the pedigree data appears to be that in families with more than one

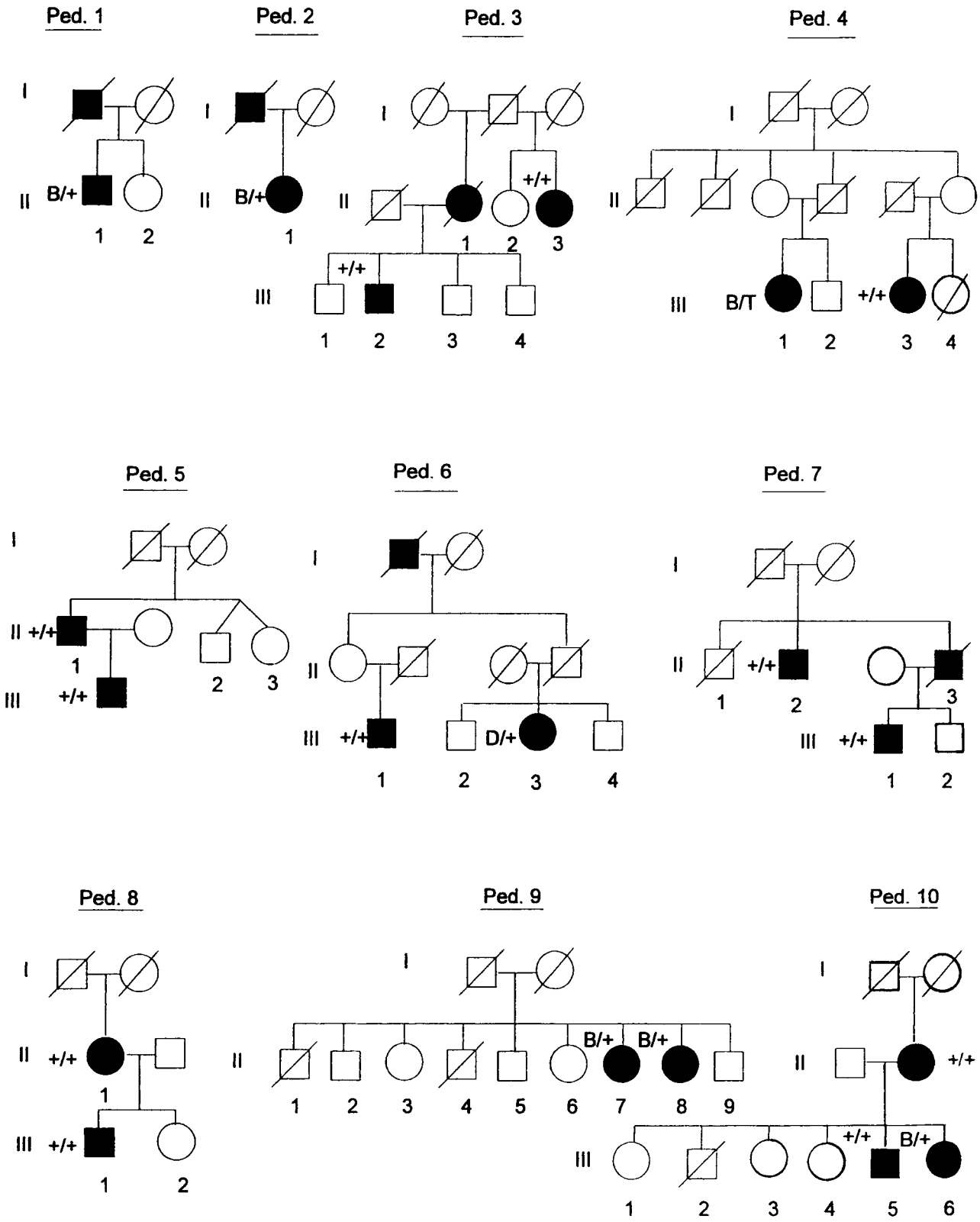

Fig. 1. Pedigrees of 10 families where parkinsonism is apparently inherited. Ped., pedigrees; squares, males; circles, females; solid symbols, definitively affected individuals; diagonal line, a deceased person; +, *wt* alleles; *B, D, T, D6* mutations.

TABLE I. Genotype Distribution for *CYP2D6* Polymorphism and Allelic Frequencies of Mutant Alleles in Parkinson Patients and Controls*

	Parkinson		Controls	
Mutant genotypes	(N = 47)	Alleles (n = 94)	(N = 47)	Alleles (n = 94)
wt/D6B	13	13	8	8
D6B/D6B	5	10	1	2
wt/D6A	1	1	0	0
wt/D6C	1	1	3	3
wt/D6D	2	2	2	2
wt/D6T	0	0	0	0
Total	22	27	14	15

* *wt*, wild-type allele; N, number of variant genotyped; n, number of variant alleles.

affected living member (pedigrees 3–10), there are either no mutant alleles, or where there are mutant alleles there are other family members with *wt* alleles (pedigrees 4, 6, and 10). The present sample of familial cases is too small to have the power to resolve differences in frequencies of PM alleles between familial and nonfamilial forms. It is difficult at present to reconcile data concerning association of mutant alleles in our population of 47 Parkinson patients and the apparent lack of tight segregation of mutant alleles among affected members within our families.

Initial support for the involvement of *CYP2D6* in Parkinson disease includes the observation that MPTP, a substrate of *CYP2D6*, induces a form of parkinsonism [Fonne-Pfister et al., 1987]; also, in the brain, *CYP2D6* is localized to the substantia nigra [Nisnik et al., 1990], an area involved in Parkinson disease; moreover, *CYP2D6* has been shown to metabolize several neurotransmitter antagonists [Eichelbaum and Gross, 1990], which raises the possibility that it may interact with some neurotransmitters, or protect against neurotransmitter antagonists [Islam et al., 1991]. Consequently, the increased risk for Parkinson disease among subjects who carry mutant *CYP2D6* alleles may be related to the reduced ability to inactivate as-yet unknown neurotoxins involved in the cause of the disease.

Tanner [1991] showed an association of the PM phenotype only in Parkinson patients with early age-at-onset, and more recently [Agúndez et al., 1995], an increased frequency of patients with genotype *wt/B* was observed, and was attributable exclusively to subjects with age-at-onset of the disease between 28–49 years. No relationship between oxidative capacity and age-at-onset of Parkinson disease was observed in British patients [Armstrong et al., 1992; Smith et al., 1992]. In contrast, our results show a slight trend toward an excess of mutant alleles in patients with Parkinson's disease when age-at-onset is ≥60 years. If real (a similar trend having been observed by Planté-Bordeneuve et al. [1994]), this finding presumably relates to the age-dependent prevalence of Parkinson disease. A larger sample will allow testing of this hypothesis (concerning direct evaluation of possible differences in relative risk for Parkinson disease in different age groups) in our laboratory.

TABLE II. Genotypic Distributions for *CYP2D6* Polymorphism in Parkinson Patients and Controls

	Homozygous	Heterozygous
Parkinson (N = 47)	5.0	17.0
Percentage	10.7	36.1
Controls (N = 47)	1.0	13.0
Percentage	2.1	27.7

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